INTEGRATED MECHANISMS OF K^+ LOSS IN MYOCARDIAL ISCHEMIA: A SIMULATION STUDY

B. Rodríguez, J. M. Ferrero (Jr)
LIB- DIE- Universidad Politécnica de Valencia (Spain)

Abstract-**During the early phase of myocardial ischemia, extracellular K^+ accumulation is considered pivotal in the genesis of reentrant arrhythmias. Despite the importance of this phenomenon, the causes of cellular K^+ loss are still unknown. Because of their ability to simulate the electrical activity of cardiomyocytes, action potential (AP) models provide an alternative for exploring their behavior under normoxic or pathological conditions. Our goal was to investigate, with the aid of computer simulations, the mechanisms responsible for the ischemic increase of extracellular K^+ concentration ($[K^+]_o$). The electrophysiological behavior of one single cardiomyocyte has been simulated during 14 minutes in the absence of coronary flow, using a modified version of Luo Rudy phase II AP model. The activation of the ATP- sensitive K^+ current, as well as other possible causes of the ischemic increase of $[K^+]_o$ such as inhibition of Na^+/K^+ pump current and altered Na^+ fluxes, have been taken into account in the simulations. Our results show that the concomitant effect of these three mechanisms in the absence of coronary flow leads to an increase of $[K^+]_o$ quantitative and qualitatively similar to the experimentally observed during acute myocardial ischemia.**

Keywords **- Ischemia, extracellular K^+ accumulation, causes.**

I. INTRODUCTION

Because of the frequent appearance of reentrant arrhythmias, the first minutes after the interruption of coronary flow are the most dangerous for cardiac tissue. The lack of oxygen and glucose leads to important changes in cellular metabolism and in the permeability of cell membranes. As a consequence, ionic concentrations are also modified and, more specifically, extracellular K^+ concentration ($[K^+]_o$) increases very early after the onset of myocardial ischemia.

Experimental reports show that the time course of $[K^+]_o$ is of triphasic nature [1]. Several seconds after the interruption of coronary flow, $[K^+]_o$ progressively increases until it reaches a plateau phase during which it remains almost constant for several minutes. Finally, a slower second rise takes place, simultaneously with a stopped glycolytic activity and cell death. The first increase of $[K^+]_o$ lasts 10-15 minutes and corresponds to the early phase of myocardial ischemia. During this period, maximal levels of $[K^+]_o$ could reach values as high as 18mM.

This extracellular K^+ accumulation is related to electrophysiological changes in cardiac tissue that have been proved to be a main cause of reentrant arrhythmias [2]. During 20 years, several experimental groups have tried to determine the precise mechanisms that make myocytes lose K^+ during acute myocardial ischemia. However, the causes of this arrhythmogenic factor remain uncertain. In this way, the simulation of action potential models could be an alternative tool to complete these investigations.

Because of its ability to simulate the electrophysiological behavior of ventricular cardiomyocytes, a modified version of Luo- Rudy phase II action potential model has been used to study the effect of several factors of myocardial ischemia on $[K^+]_o$ in one single ventricular myocyte. The activation of the ATP- sensitive K^+ current ($I_{K(ATP)}$), the inhibition of Na^+/K^+ pump current (I_{NaK}) or an enhanced Na^+ influx have been proposed to be the most probable among the possible causes of cellular K^+ loss during ischemia [1, 3]. The goal of this work was to determine the effect of ischemia- induced changes in these three mechanisms of ionic transport on $[K^+]_o$ in one single ventricular myocyte in the absence of coronary flow.

II. METHODOLOGY

A modified version of Luo- Rudy phase II action potential model [4] has been used to simulate the electrophysiological behavior of one single cardiomyocyte, during 14 minutes of interrupted coronary flow. Four factors of myocardial ischemia have been considered in our simulations and their time course is described in Fig. 1.

A formulation of $I_{K(ATP)}$ was incorporated into the model [5]. To simulate its activation during ischemia, the fraction of opened ATP- dependent K^+ channels (f_{ATP}) was increased in a linear manner from zero to a final value of 0.84% during 14 minutes of interrupted coronary flow.

Inhibition of Na^+/K^+ pump activity was simulated by substituting the maximal current (I_{NaKMAX}) by the expression:

$$I_{NaKMAX}(t) = I_{NaKMAX\_INI} \cdot [1 - f_{INHIB}(t)] \quad (1)$$

where I_{NaKMAX_INI} is 2.75μA/μF (the normoxic value of I_{NaKMAX}) [4] and $f_{\_INHIB}$ is the inhibition degree of Na^+- K^+ pump. $f_{\_INHIB}$ progressively increased from zero to its final value 35%, in accordance with experimental observations [6].

During myocardial ischemia, altered Na^+ fluxes could lead to a Na^+ leak current [3], represented in our simulations by the new Na^+ current (I_{ONa}). The amplitude of this current was linearly increased from zero to its final value –1.2μA/μF. In accordance with experimental results, its activation begins two minutes later than the changes in $I_{K(ATP)}$ and in I_{NaK} [7,8].

The onset of myocardial ischemia leads to the absence of ionic flow between the interstitial space (cleft) and the bulk extracellular medium. This phenomenon was simulated by steeply increasing the time constant for diffusion of ions (τ_{diff}) from its normoxic value 1000ms to 10^8 ms, as described in Fig. 1. In order to stabilize ionic concentrations, τ_{diff} was increased one minute before the beginning of alterations in $I_{K(ATP)}$ and in I_{NaK}.

Report Documentation Page

Report Date	Report Type	Dates Covered (from... to)
25 Oct 2001	N/A	-

Title and Subtitle Integrated Mechanisms of K Loss in Myocardial Ischemia: A Simulation Study	**Contract Number**
	Grant Number
	Program Element Number
Author(s)	**Project Number**
	Task Number
	Work Unit Number
Performing Organization Name(s) and Address(es) LIB-DIE-Universidad Politecnica de Valencia Spain	**Performing Organization Report Number**
Sponsoring/Monitoring Agency Name(s) and Address(es) US Army Research, Development & Standardization Group (UK) PSC 802 Box 15 FPO AE 09499-1500	**Sponsor/Monitor's Acronym(s)**
	Sponsor/Monitor's Report Number(s)

Distribution/Availability Statement
Approved for public release, distribution unlimited

Supplementary Notes
Papers from 23rd Annual International Conference of the IEEE Engineering in Medicine and Biology Society, October 25-28, 2001, held in Istanbul, Turkey. See also ADM001351 for entire conference on cd-rom.

Abstract

Subject Terms

Report Classification unclassified	**Classification of this page** unclassified
Classification of Abstract unclassified	**Limitation of Abstract** UU

Number of Pages
4

To investigate the effect of simulated ischemia on unidirectional K^+ fluxes, we monitored the number of K^+ ions transported into and out of the cell, n_{IKout} and n_{INaK} respectively, which are defined as:

$$n_{IKout}(t) = \int_0^t I_{Kout} d\hat{o} \quad (2)$$

$$n_{INaK}(t) = \int_0^t I_{NaK} d\hat{o} \quad (3)$$

Then, $divn_{IKout}(t)$ and $divn_{InaK}(t)$ were computed as:

$$divn_{IKout}(t) = \frac{n_{IKout}(t) - n_{IKout}(60s)}{n_{IKoutNORM}(t) - n_{IKoutNORM}(60s)} \quad (4)$$

$$divn_{INaK}(t) = \frac{n_{INaK}(t) - n_{INaK}(60s)}{n_{NaKNORM}(t) - n_{INaKNORM}(60s)} \quad (5)$$

where $n_{IKoutNORM}(t)$ and $n_{INaKNORM}(t)$ are, respectively, $n_{IKout}(t)$ and $n_{INaK}(t)$ under normoxic conditions but in the absence of coronary flow

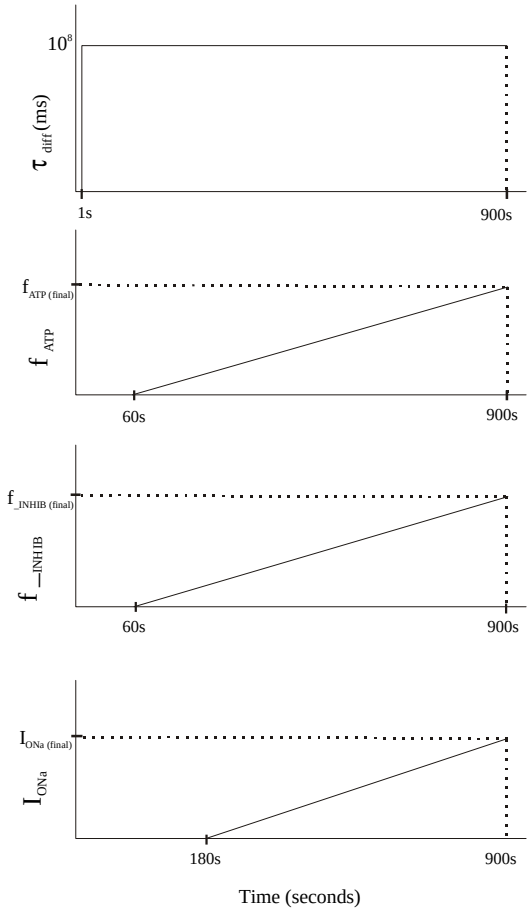

Fig. 1. Time course of ischemia- induced changes in ionic currents.

The model used in the simulations considers the three following compartments: the intracellular space, the interstitial extracellular clefts and a bulk extracellular medium in which concentrations were assumed to be constant. Dynamic changes in the extracellular (cleft) concentration of each ionic specie S ($[S]_o$) are simulated with the equation [5]:

$$\frac{d[S]_o}{dt} = -\frac{A_m}{V_{cleft} F} I_{S,tot} - \frac{[S]_o - [S]_{bulk}}{\hat{o}_{diff}} \quad (6)$$

where A_m is the area of the myocyte, V_{cleft} is the volume of the interstitial cleft (per cell), F is the Faraday constant, $I_{S,tot}$ is the total ionic current associated to ion S, τ_{diff} is the time constant for diffusion of S from the interstitial clefts to the bulk extracellular medium, and $[S]_{bulk}$ is the S concentration in the bulk.

The frequency of stimulation was 90 bpm that corresponds to a basic cycle length (BCL) of 666ms. The model was written in ACSL language. The nonlinear system of different equations was solved using the Gear stiff method. A maximum time step of 0.01ms was allowed.

III. Results

A. Extracellular K^+ accumulation during simulated ischemia.

As specified above, ischemia- induced alterations of three mechanisms of ionic transport ($I_{K(ATP)}$, I_{NaK} and I_{ONa}) have been proposed to be at least in part responsible for cellular K^+ loss during the first minutes of interrupted coronary flow. Computer simulations have been carried out to determine the contribution of these mechanisms to extracellular K^+ accumulation. Fig. 2 summarizes the results of four different cases. Simulations A, B and C correspond to the activation of only one of the ischemic mechanisms in the absence of coronary flow, while simulation D considers the concomitant effect of the alteration of the three currents.

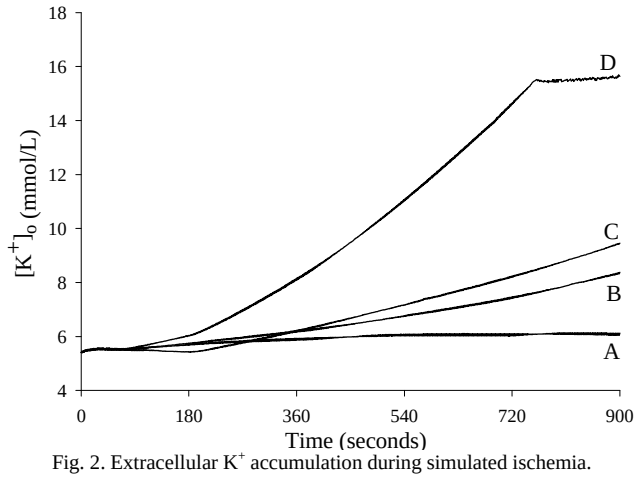

Fig. 2. Extracellular K^+ accumulation during simulated ischemia.

Simulation A represents the slight increase of $[K^+]_o$ during the progressive opening of K_{ATP} channels until f_{ATP} reaches a value of 0.84%. The final $[K^+]_o$ is about 0.8mmol/L above its normoxic value (5.4mmol/L). Simulation B describes the time course of $[K^+]_o$ for 14 minutes during which I_{NaKMAX} is decreased to 65% of its normoxic value. An extracellular K^+ accumulation progressively takes place until a final $[K^+]_o$ of 8mmol/L is reached. Finally, as shown in simulation C, the linear activation of I_{ONa} to -1.2μA/μF leads to a final $[K^+]_o$ of 9.8mmol/L. Experimental reports show that the first increase of $[K^+]_o$ is followed by a plateau phase with a final level that could reach 18mM [1]. Our simulations prove that the activation of only one of these mechanisms is not able to reproduce this extracellular K^+ accumulation, neither quantitatively nor qualitatively.

Simulation D shows the integrated effect of the three mechanisms on $[K^+]_o$ during 14 minutes of interrupted washout. The activation of $I_{K(ATP)}$ simultaneous with Na^+/K^+ pump inhibition is followed by a first rise of $[K^+]_o$, which is enhanced by the increase of I_{ONa}. Then, 11.6 min later, $[K^+]_o$ reaches a plateau level of 15.5mmol/L and remains almost constant for the rest of the 14 minutes simulated.

B. Unidirectional K^+ fluxes during acute simulated ischemia.

Fig. 2 shows that only the integrated effect of changes in $I_{K(ATP)}$, I_{NaK} and I_{ONa} leads to an increase of $[K^+]_o$ similar to the experimentally observed during acute myocardial ischemia. In order to further investigate the causes of this cellular K^+ loss, we have monitored the time course of $divn_{INaK}$ and $divn_{Ikout}$, both defined in Methods. These mathematical variables compare the number of K^+ ions transported across the cell membrane during simulated ischemia with ionic fluxes under normoxic conditions. Thus, they provide interesting information about the consequences of simulated ischemia on unidirectional K^+ fluxes. If $divn_{INaK}$, or $divn_{IKout}$ is higher than one, then K^+ influx or K^+ efflux respectively has being reduced by the effect of the ischemic mechanisms considered. Fig. 3 depicts the time course of $divn_{INaK}$ and $divn_{IKout}$ under the conditions of simulated ischemia described in Fig. 1.

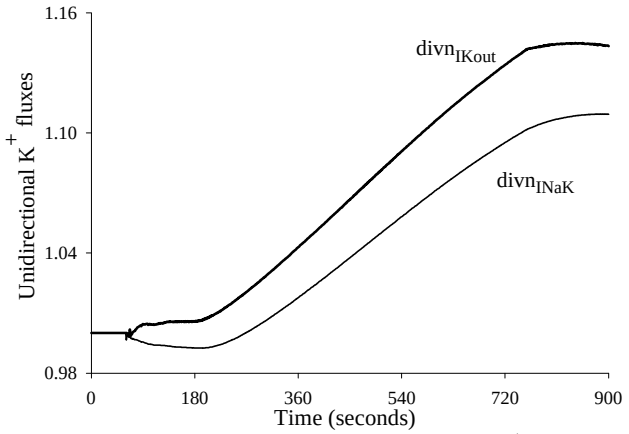

Fig. 3. Effect of simulated ischemia on unidirectional K^+ fluxes.

As it can be observed, one minute after the interruption of coronary flow, the simultaneous effect of changes in $I_{K(ATP)}$ and I_{NaK} produces a slight increase of $divn_{IKout}$. Two minutes later , the activation of I_{ONa} provokes a faster increase of $divn_{IKout}$ that denotes an important enhancement of outward K^+ current. During all the simulation, $divn_{IKout}$ is higher than one, proving that the concomitant alteration of $I_{K(ATP)}$, I_{NaK} and I_{ONa} in the absence of coronary flow increases unidirectional outward K^+ flux. Three minutes before the end of the simulation, the slope of the curve changes and $divn_{IKout}$ stabilizes in its final value 1.15. On the other hand, the time course of $divn_{INaK}$ shows that, during the first two minutes of simulated ischemia, the linear decrease of I_{NaKMAX} reduces the ability of Na^+/K^+ pump to transport K^+ ions into the cell. Subsequently, a few seconds after the activation of I_{ONa}, $divn_{INaK}$ progressively increases up to 1, showing an enhancement of K^+ influx despite the reduction of I_{NaKMAX}. As for $divn_{IKout}$, the slope of the curve decreases and at the end of the simulation the final value of $divn_{INaK}$ was 1.10.

IV. Discussion

Experimental reports have proved that extracellular K^+ accumulation is in the origin of reentrant arrhythmias during acute myocardial ischemia. The importance of this phenomenon has involved a large number of investigators in the determination of the causes of cellular K^+ loss. Even if the precise mechanisms are still unclear, their results suggest that opening of K_{ATP} channels, inhibition of Na^+/K^+ pump activity and altered Na^+ fluxes could play an important role in the ischemic increase of $[K^+]_o$.

In accordance with precedent studies from our group [9, 10, 11], our simulations show that the separate activation of only one of these mechanisms is not able to cause the extracellular K^+ accumulation experimentally observed. However, their simultaneous alteration leads to a progressive rise of $[K^+]_o$ followed by a plateau phase during which $[K^+]_o$ remains almost constant. Qualitatively, this biphasic time course of $[K^+]_o$ is typical of the first 10-15 minutes of myocardial ischemia [1]. Quantitatively, the rate of rise of $[K^+]_o$ and the plateau level are in the range of those measured in cardiac tissues of pig, rabbit and guinea-pig. In conclusion, the simultaneous effect of the progressive change of $I_{K(ATP)}$, I_{NaK} and I_{ONa} in the absence of coronary flow accurately reproduces the extracellular K^+ accumulation observed during the early phase of mycardial ischemia.

During the first two minutes of simulated ischemia, the opening of K_{ATP} channels represents an increase of K^+ permeability of cell membrane that could enhance K^+ efflux and extracellular K^+ accumulation. However, our results show that the single activation of $I_{K(ATP)}$ produces a final extracellular K^+ accumulation of 0.8mmol/L. A possible explanation of this small effect is that the opening of K_{ATP} channels increases K^+ efflux across $I_{K(ATP)}$ but also produces a faster repolarization of action potential. As a consequence, action potential duration is shortened and the time during which other K^+ currents are activated is reduced. Furthermore, the $I_{K(ATP)}$ enhancement of g_K not only produces

the shortening of APD but also a reduction of K^+ driving force that would restrain outward K^+ fluxes. Thus, extracellular K^+ accumulation during ischemia could only be explained if this self- limiting effect of g_K is counteracted by an inward current.

The nature of this inward current is debated but experimental evidences have suggested that altered Na^+ fluxes could contribute to cellular K^+ loss [12]. In this way, the accumulation of substances like lysophosphatidylcoline has been proved to profoundly modify the characteristics of Na^+ channels [13]. Their action during ischemia could result in a net Na^+ inward current that could contribute to the increase of $[Na^+]_i$ [7]. Simultaneously, this depolarizing current would enhance K^+ driving force and cellular K^+ loss during acute myocardial ischemia.

In accordance with this hypothesis, our results show that an inward current is necessary to explain the ischemic K^+ accumulation in the extracellular space. The activation of I_{ONa} counteracts the effects of increasing f_{ATP} on APD and on K^+ driving force and facilitates cellular K^+ loss across $I_{K(ATP)}$ and other K^+ channels. Then, the concomitant effect of the opening of K_{ATP} channels and the activation of a Na^+ inward current provokes a fast increase of unidirectional outward K^+ flux.

In addition to the increase of K^+ efflux, a decrease of Na^+/K^+ pump ability to transport K^+ ions would contribute to their accumulation in the extracellular space. In this way, experimental reports suggest that the pump could be partially inhibited by several ischemic factors like accumulation of inorganic phosphates, decreased intracellular pH and reduced intracellular ATP levels. However, other studies have shown that K^+ influx persists and even is enhanced after the interruption of coronary flow [1].

Our results could explain this apparent discrepancy because, even if the I_{NaKMAX} is linearly decreased, K^+ influx is only slightly reduced during the first minutes of simulated ischemia. Once the activation of I_{ONa} begins, the increase of $[Na^+]_i$ and also of $[K^+]_o$ is sufficient to enhance I_{NaK} despite the reduction of I_{NaKMAX}. Thus, in agreement with experimental observations, Na^+/K^+ pump activity could play an important role on the onset of the plateau phase.

V. Conclusion

The biphasic time course of $[K^+]_o$ observed during the first 14 minutes of simulated ischemia is quantitative and qualitatively in accordance with the experimental data obtained during the acute phase of myocardial ischemia.

These results support the hypothesis that extracellular K^+ accumulation could be caused by the concomitant effect of opening of K_{ATP} channels, Na^+/K^+ pump inhibition and a net Na^+ inward current in the absence of coronary flow. Cellular K^+ loss results from an increase of unidirectional K^+ efflux which is counteracted by an enhancement of Na^+/K^+ current, possibly produced by changes in ionic concentrations.

References

[1] A. Wilde, G. Aksnes, "Myocardial potassium loss and cell depolarization in ischemia and hypoxia," *Cardiovasc. Res*, vol. 29, pp. 1-15, 1995.

[2] AS. Harris, A. Bisteri, RA. Russel, JC. Brigham, JE. Firestone, "Excitatory factors in ventricular tachycardia resulting from myocardial ischemia. Potassium a major excitant," Science, vol.119, pp. 200-203, 1954.

[3] E. Carmeliet, "Cardiac ionic currents and acute ischemia: from channels to arrhythmias," *Physiol. Rev*, vol.79, pp. 917-1017, 1999.

[4] CH. Luo, YA. Rudy, "A dynamic model of the cardiac ventricular action potential. 1. Simulations of ionic currents and concentration changes," *Circ. Res*, vol. 74, pp. 1071-1096, 1994.

[5] JM. Ferrero (Jr), J. Sáiz, JM. Ferrero, N. Thakor, "Simulation of action potentials from metabolically impaired cardiac myocytes. Role of the ATP- sensitive K current," *Circ. Res*, vol. 68, pp. 208-221, 1996.

[6] MM. Bersohn, KD. Philipson, JY. Fukushima, "Sodium-calcium exchange and sarcolemmal enzymes in ischemic rabbit hearts," *Am. J. Physiol*, vol. 242, pp. C288-C295, 1982.

[7] U. Decking, M. Hartmann, H. Rose, R. Brückner, J. Meil, "Cardioprotective actions of KC12291 I. Inhibition of voltage-gated Na^+ channels in ischemia delays myocardial Na^+ overload," *Naunyn- Schmiedeberg. Arch. Pharmacol*, vol. 358, pp. 547-553, 1998.

[8] YK. Ju, DA. Saint, PW. Gage, "Hypoxia increases persistent sodium current in rat ventricular myocytes," *J. Physiol*, vol. 497.2, pp. 337-347, 1996.

[9] B. Rodríguez, JM. Ferrero (Jr), B. Trénor, JM. Ferrero, J. Sáiz, NV. Thakor, " Simulation study of the contribution of ATP-dependent potassium current to extracellular potassium accumulation during myocardial ischemia," *IEEE/ EMBS, Hong Kong*, vol. 20, pp. 174-177, 1998.

[10] B. Rodríguez, JM. Ferrero (Jr), "Effect of Na^+ inward current on extracellular K^+ accumulation during myocardial ischema. A simulation study," *IEEE XXVI Computers in Cardiology, Hannover*, vol. 26, pp. 101-103, 1999.

[11] B. Rodríguez, JM. Ferrero (Jr), "Effect of Na^+/K^+ pump inhibition on extracellular potassium accumulation during myocardial ischemia: a simulation study," *IEEE XXVII Computers in Cardiology, Cambridge*, vol. 27, pp. 315-318, 2000.

[12] K. Shivkumar, N. Deutsch, ST. Lamp, K. Khuu, J. Goldhaber, J. Weiss, "Mechanisms of hypoxic K loss in rabbit ventricle," *J. Clin. Invest*, vol. 100, pp. 1782-1788, 1997.

[13] A. Undrovinas, I. Fleidervish, J. Makielski, "Inward current at resting potentials in single cardiac myocytes induced by the ischemic metabolite lysophosphatidylcholine," *Circ. Res*, vol. 71, pp. 1231-1241, 1992.